TITLE
Narp regulates homeostatic scaling of excitatory synapses on Parvalbumin interneurons

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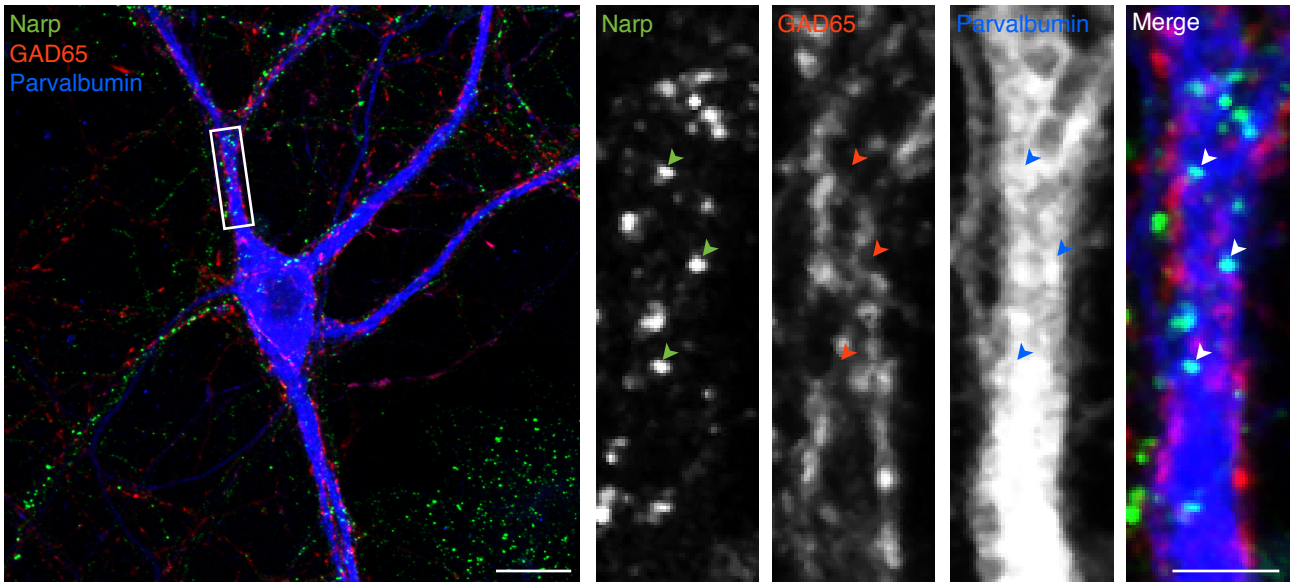
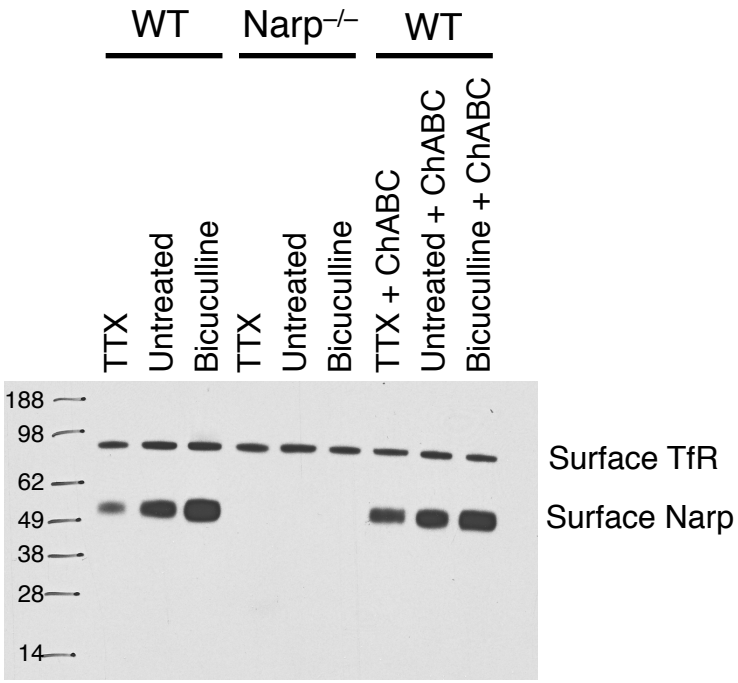
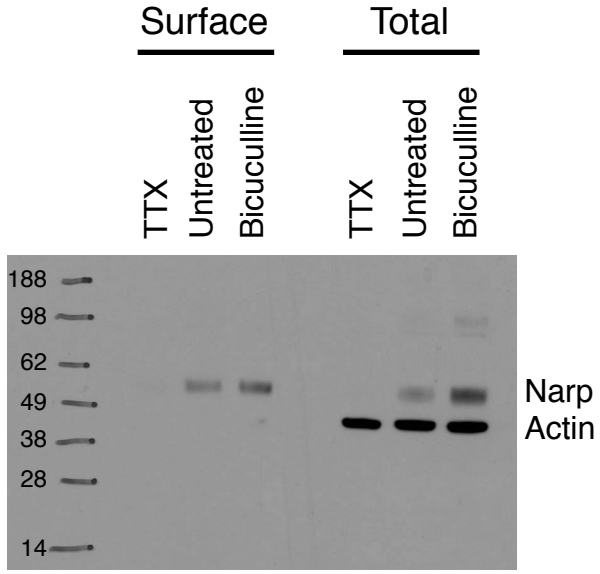
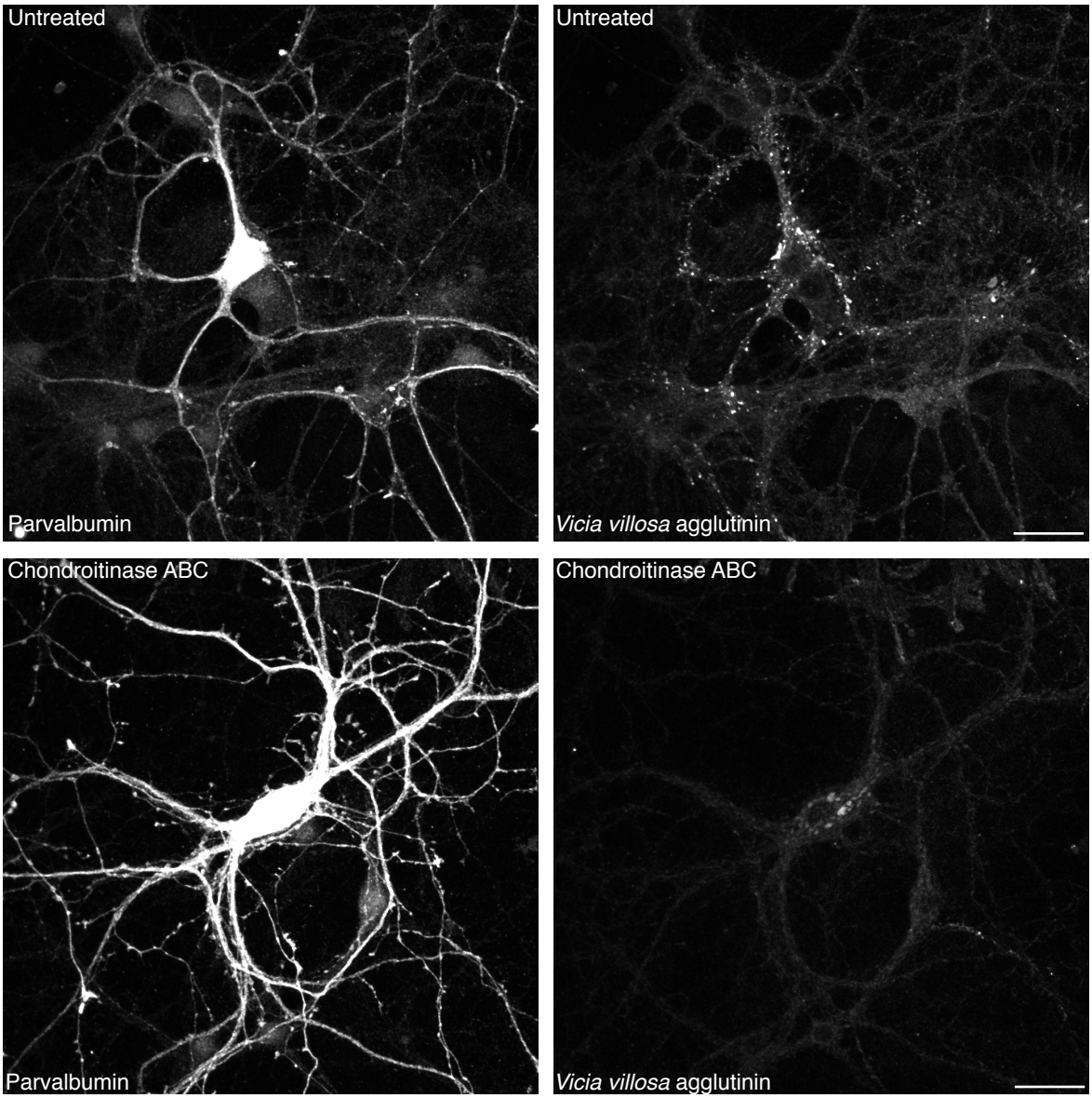


Supplementary figure 1. Narp does not localize to inhibitory synapses. Cultured hippocampal neurons were labeled using antibodies against Narp (green), GAD65 (red), and Parvalbumin (blue). Arrowheads show Narp puncta not colocalized with GAD65. Scale bars represent 10 µm or 2.5 µm (inset).

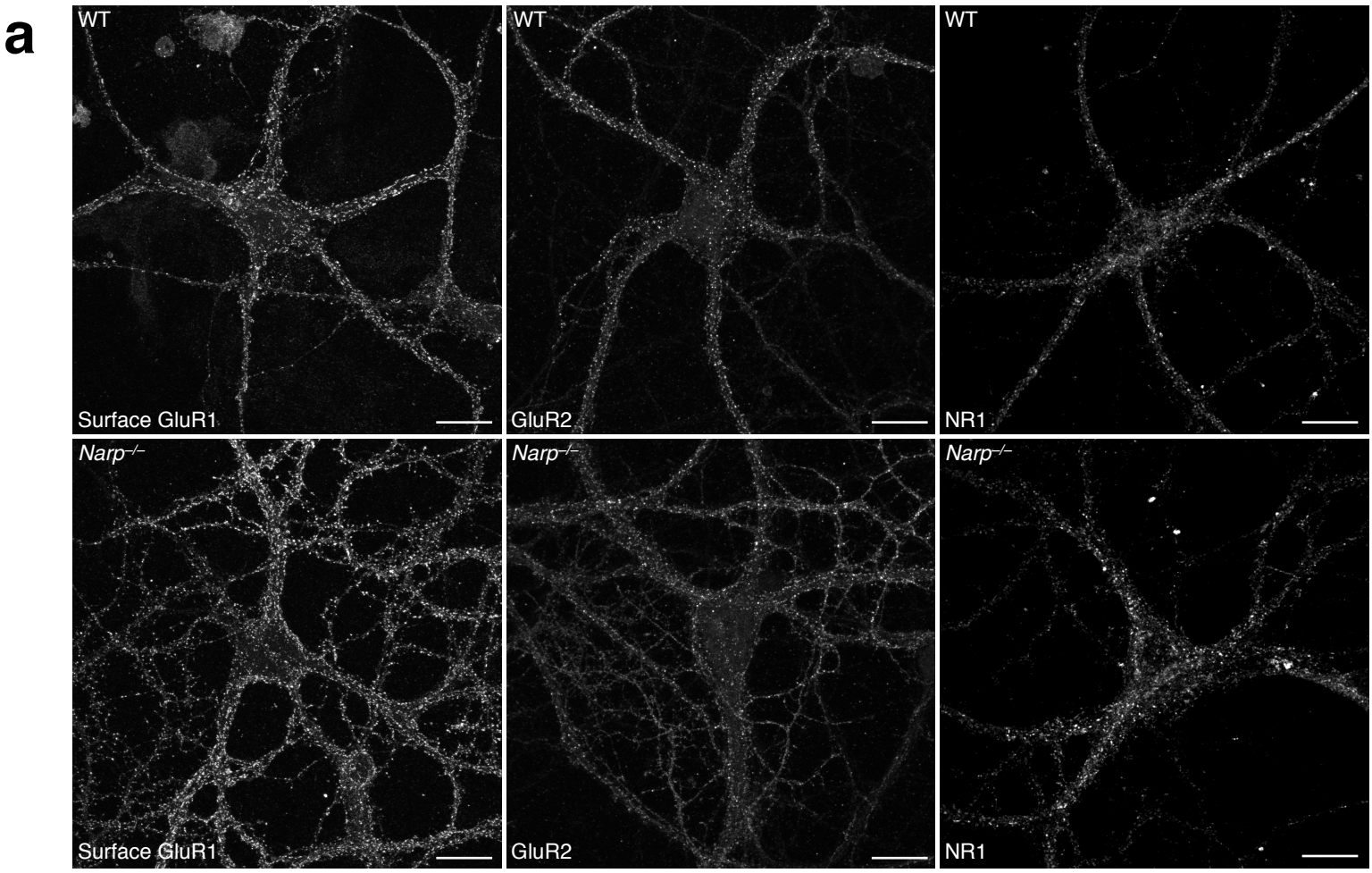




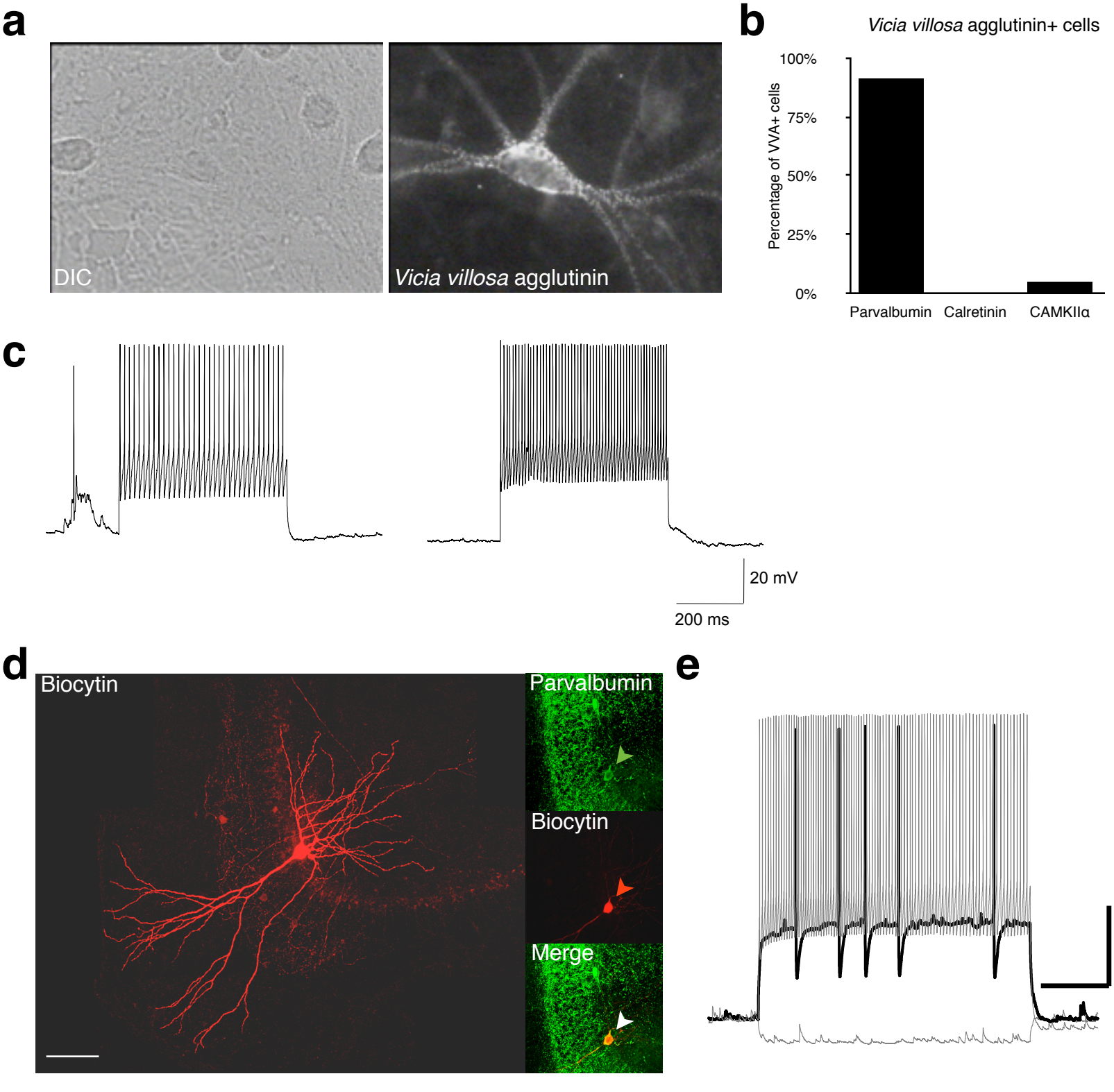
Supplementary Figure 2. Full-length blots showing Narp, TfR, and Actin levels under different conditions. Samples from both blots derived from the same experiment.



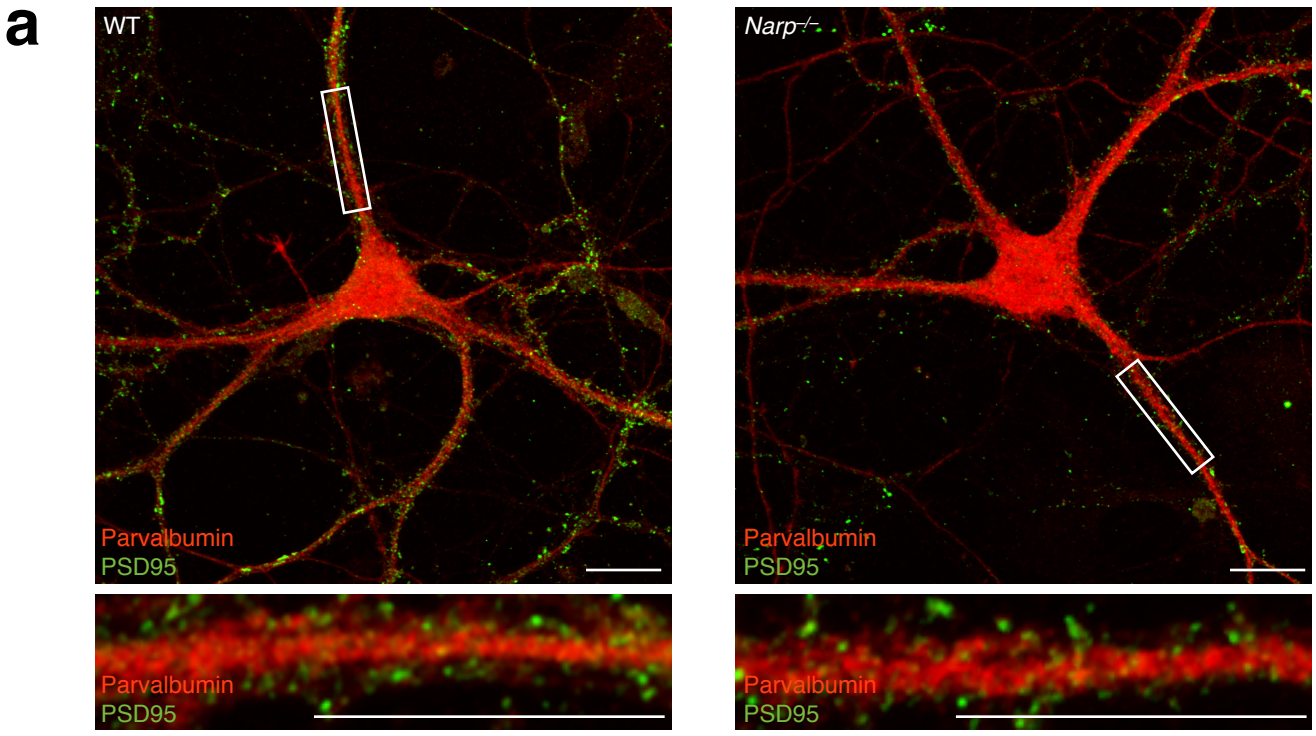
Supplementary figure 3. Effect of chondroitinase ABC on perineuronal nets. Cultured neurons were treated with either control (top panels) or chondroitinase ABC (bottom panels) for 48 hours prior to live labeling with fluorescein-conjugated *Vicia villosa* agglutinin (right panels) and subsequent Parvalbumin staining (left panels). Scale bars represent 10 µm.

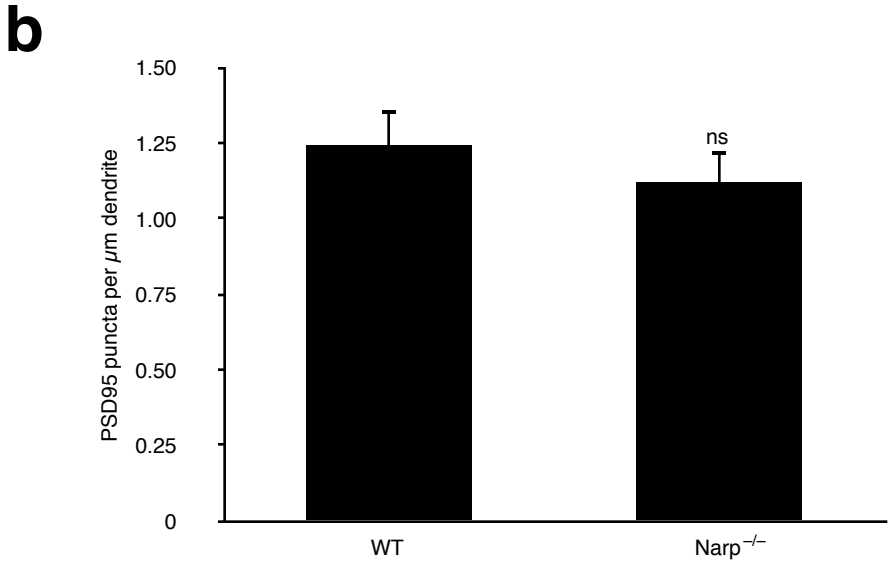


Supplementary figure 4. Other glutamate receptor levels are not altered in *Narp*$^{-/-}$ PV-INs. (**a**) Representative images of WT (top) and *Narp*$^{-/-}$ (bottom) cultured hippocampal neurons that were live labeled with antibodies against GluR1 (left), GluR2 (middle), or NR1 (right). Scale bars represent 10µm. (**b**) Summary of the data shown in **a**. GluR1, GluR2, or NR1 intensity per µm dendrite was normalized to respective WT PV-IN group (WT GluR1, 100% ± 9.28%, n = 11 cells; *Narp*$^{-/-}$ GluR1, 118.44% ± 13.72%, n = 12 cells; WT GluR2, 100% ± 10.85%, n = 15 cells; *Narp*$^{-/-}$ GluR2, 107.85% ± 13.73%, n = 15 cells; WT NR1, 100% ± 9.60%, n = 15 cells; *Narp*$^{-/-}$ NR1, 94.90% ± 13.32%, n = 15 cells). Statistical analysis was performed using an unpaired t-test with Welch's correction. Error bars represent s.e.m.


a
DIC
Vicia villosa agglutinin
b
Vicia villosa agglutinin+ cells
Percentage of VVA+ cells
100%
75%
50%
25%
0%
Parvalbumin
Calretinin
CAMKIIα
c
20 mV
200 ms
d
Biocytin
Parvalbumin
Biocytin
Merge
e

Supplementary figure 5. Parvalbumin interneuron identification (**a**) PV-INs in dissociated hippocampal cultures are initially identified based on binding of fluoroscein-conjugated *Vicia villosa* agglutinin (VVA) (right panel). (**b**) Hippocampal neuronal cultures were co-stained with VVA and either Parvalbumin, Calretinin, or CAMKIIα. VVA binds almost exclusively to cells expressing Parvalbumin (Parvalbumin, 91.40%, n = 93 cells; Calretinin, 0%, n = 100 cells; CAMKIIα, 4.84%, n = 124 cells) (**c**) After visual identification of PV-INs in the culture they were recorded in whole-cell patch clamp configuration and PV-INs were confirmed via their non-accommodating, fast-spiking behavior in response to current injection (left, 750 pA; right, 1500 pA). (**d**) PV-IN recorded in whole-cell mode in the dentate gyrus of an acute hippocampal slice was injected with biocytin and recovered post-hoc with Alexa-543 conjugated avidin (red). The tissue was also processed for PV immunoreactivity as shown in the insets (green) and the recorded cell was revealed to be PV+. Scale bar represents 60 µm. (**e**) Membrane potential of the recorded PV-IN from **d** in response to a hyperpolarizing (–200 pA), just threshold depolarizing (400 pA, thick black trace) and twice threshold depolarizing (800 pA) current injection (bars 200 ms/20 mV). Consistent with PV-IN morphology/expression, the cell exhibits typical fast-spiking behavior. Note this cell corresponds to the sEPSC recording for WT MECS in Fig. 7a.





Supplementary Figure 6. Synapse numbers are not altered in *Narp*$^{-/-}$ PV-INs. (**a**) Representative images of WT (left) and *Narp*$^{-/-}$ (right) cultured hippocampal neurons that were labeled with antibodies against PSD95 (green) and PV (red). Scale bars represent 10 µm. (**b**) Summary of the data shown in **a**. Number of PSD95-positive puncta per µm dendrite in WT and *Narp*$^{-/-}$ PV-INs (WT, 1.24 ± 0.12, n = 15 cells; *Narp*$^{-/-}$, 1.12 ± 0.11, n = 15 cells). Statistical analysis was performed using an unpaired t-test with Welch's correction. Error bars represent s.e.m.

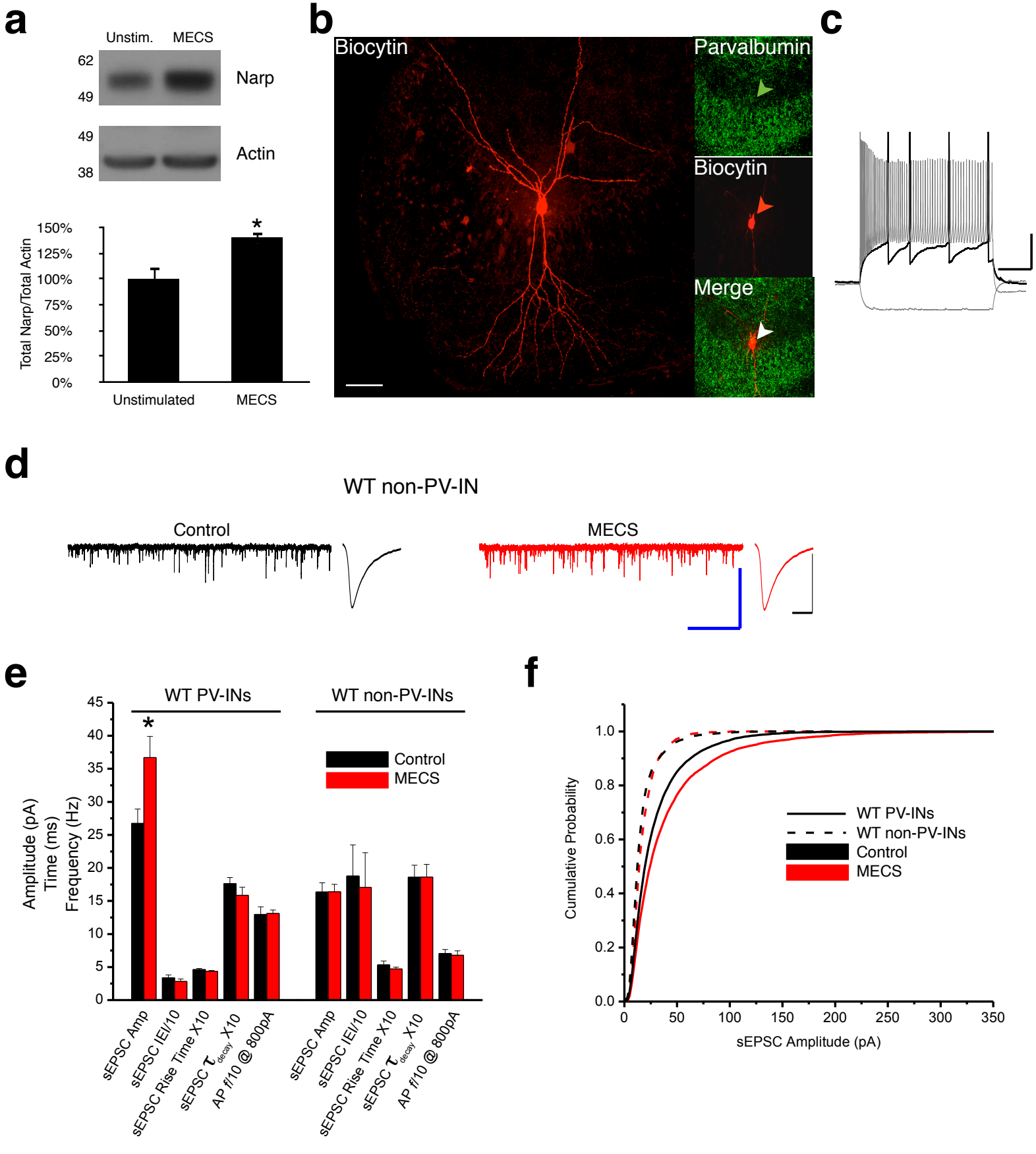
a

	WT (n = 12)		*Narp*$^{-/-}$ (n = 22)	
	Mean	s.e.m.	Mean	s.e.m.
R_{input} (MΩ)	71.0631	11.4008	67.6970	6.8494
RMP (mV)	–63.7951	1.5200	–63.0289	1.4189
AP Amplitude (mV)	56.5408	2.1429	54.8984	1.7610
AP Frequency (Hz)	2.5239	0.5679	1.2025**	0.1858
AP max rise slope (mV/ms)	115.4170	7.7517	117.2611	4.4698
AP max decay slope (mV/ms)	–85.9321	8.3758	–85.2962	4.1609
t_{peak} -> t_{decay} (ms)	0.4417	0.0452	0.3864	0.0192
t_{rise} -> t_{decay} (ms)	0.7919	0.0722	0.6955	0.0275

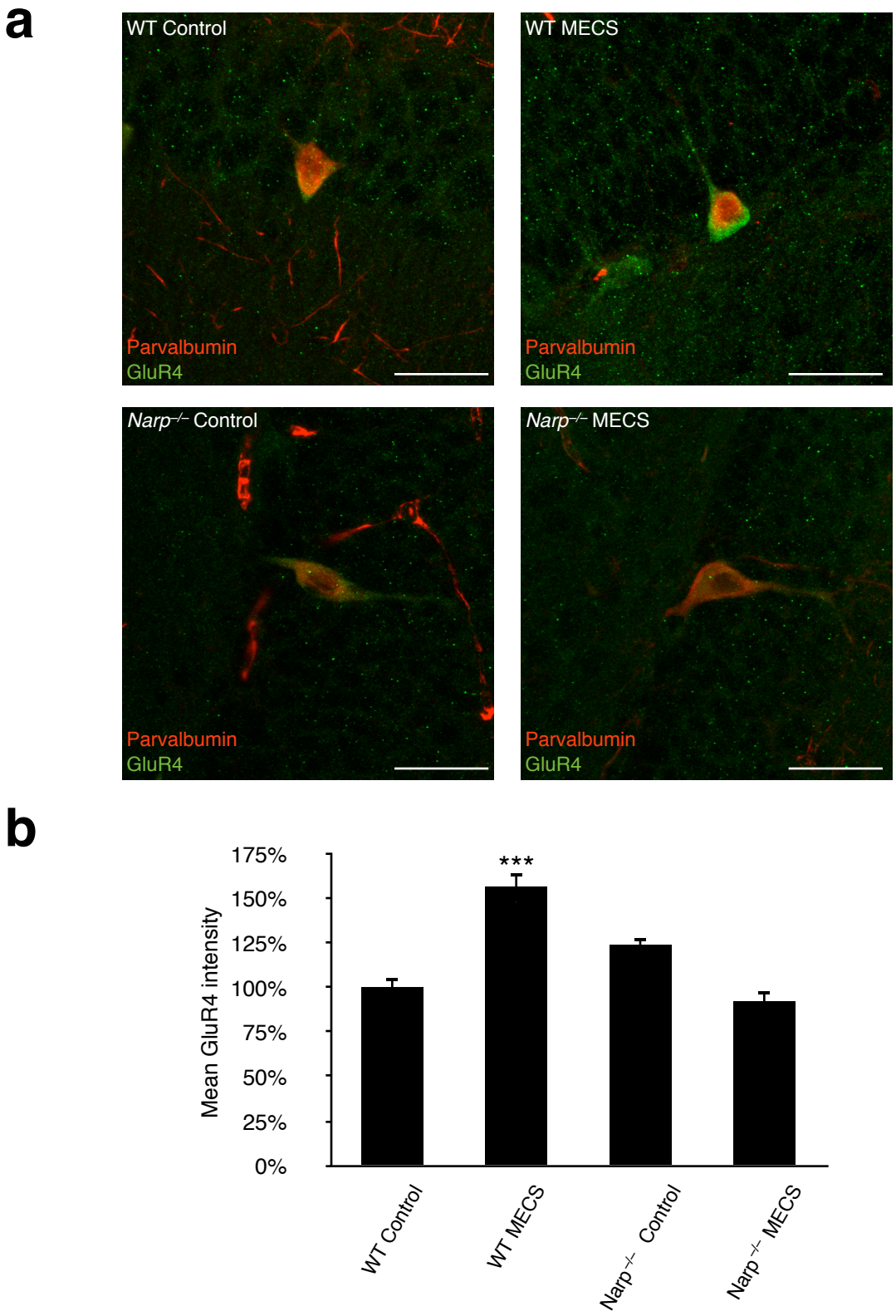
b

	WT		*Narp*$^{-/-}$	
	Mean (Hz)	s.e.m.	Mean (Hz)	s.e.m.
TTX	108.8416 (n = 17)	6.8478	108.0656 (n = 13)	5.5128
Untreated	107.2759 (n = 20)	3.9836	108.9856 (n = 23)	4.0562
Bicuculline	108.6422 (n = 15)	5.3426	108.4011 (n = 13)	8.2540

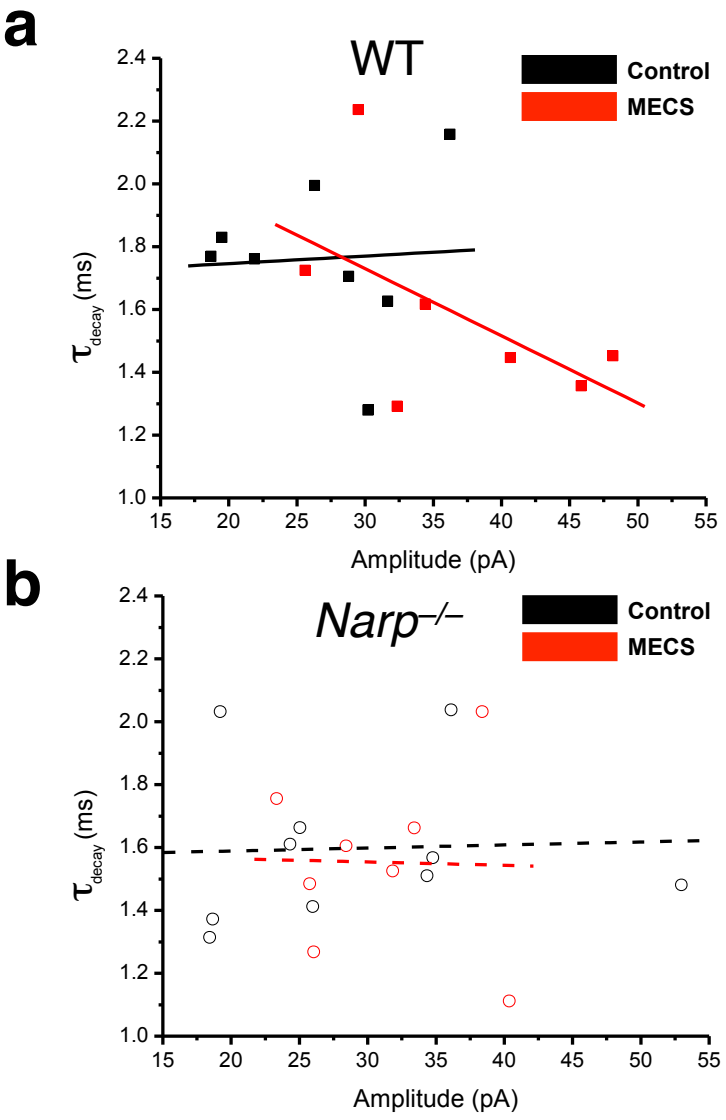
Supplementary Figure 7. PV-IN physiological properties (**a**) Summary of mean membrane and action potential properties of cultured PV-INs (t_{peak} - time of action potential peak, t_{decay} - beginning of the maximum decay slope t_{rise} - beginning of the maximum rise slope) (**b**) Average maximal firing rates in response to 500 ms, 1500 pA sustained current injection.


a
Unstim.
MECS
62
49
Narp
49
38
Actin
Total Narp/Total Actin
150%
125%
100%
75%
50%
25%
0%
*
Unstimulated
MECS
b
Biocytin
Parvalbumin
Biocytin
Merge
c
d
WT non-PV-IN
Control
MECS
e
WT PV-INs
WT non-PV-INs
Amplitude (pA)
Time (ms)
Frequency (Hz)
45
40
35
30
25
20
15
10
5
0
*
Control
MECS
sEPSC Amp
sEPSC IEI/10
sEPSC Rise Time X10
sEPSC τdecay X10
AP f/10 @ 800pA
sEPSC Amp
sEPSC IEI/10
sEPSC Rise Time X10
sEPSC τdecay X10
AP f/10 @ 800pA
f
Cumulative Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
350
sEPSC Amplitude (pA)
WT PV-INs
WT non-PV-INs
Control
MECS

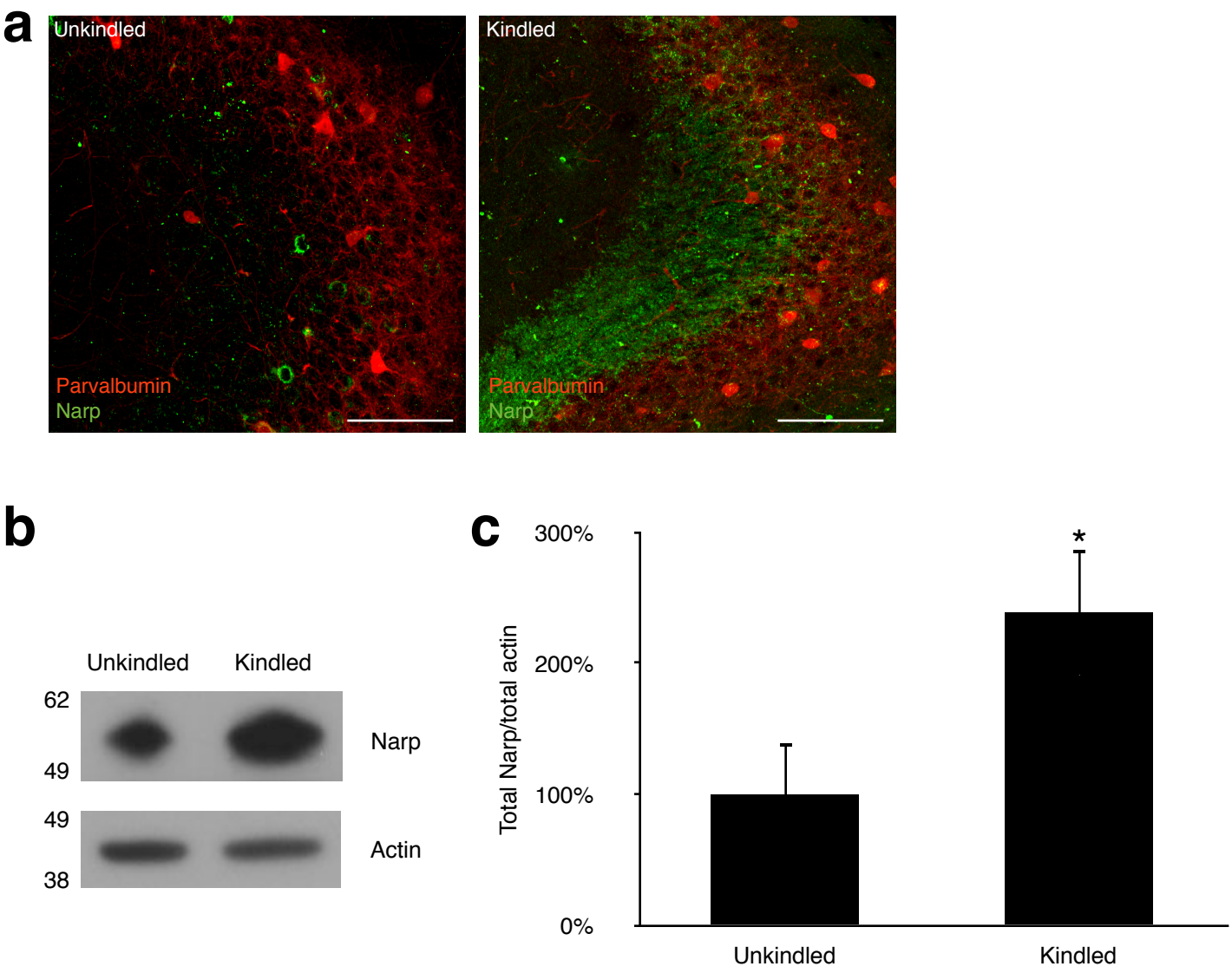
Supplementary figure 8. MECS does not alter sEPSC properties in non-PV interneurons of the dentate gyrus. (**a**) (top) Representative western blot showing total levels of Narp and Actin in unstimulated control (left) and 16 hours post-MECS (right) in mouse forebrain. (bottom) Summary of the data shown at top. All values are presented as a ratio of total Narp intensity/total Actin intensity and were normalized to unstimulated control (Unstimulated, 100% ± 12.70%, n = 3 mice; MECS, 140.28% ± 5.84%, n = 3 mice). Statistical analysis was performed using a Student's t-test. * $P < 0.05$. Error bars represent s.e.m. (**b**) Representative non-PV interneuron (non-PV-IN) recorded in whole-cell mode in the dentate gyrus of an acute hippocampal slice that was injected with biocytin and recovered post-hoc with Alexa-543 conjugated avidin (red). The tissue was also processed for PV immunoreactivity as shown in the insets (green) and the recorded cell was revealed to be PV negative. Scale bar represents 60 µm. (**c**) Membrane potential of the recorded non-PV-IN from **b** in response to a hyperpolarizing (–200 pA), just threshold depolarizing (100 pA, thick black trace) and six times threshold depolarizing (600 pA) current injection (bars 200 ms/20 mV). (**d**) Representative sEPSC records of non-PV-INs in acute slices from control (left) and MECS treated (right) WT mice (bars 1 s/100 pA). At right of each trace is also shown the average sEPSC from each record (bars 2 ms/20 pA). Note that the cell illustrated in **b** corresponds to the MECS sEPSC record. (**e**) Bar chart summary of average sEPSC amplitudes, interevent intervals (IEIs), rise times, and decay time constants obtained from recordings in WT PV-INs (replotted from Fig 7e) and WT non-PV-INs from control and MECS treated mice as indicated. Also shown is the group data for action potential frequency observed in response to a sustained current injection (0.8 s/800 pA) in current-clamp mode (PV-IN Control sEPSC amplitude, 26.7 pA ± 2.2 pA, n = 8 cells from 4 mice; PV-IN MECS sEPSC amplitude, 36.7 pA ± 3.2 pA, n = 7 cells from 4 mice; non-PV-IN Control sEPSC amplitude, 16.4 pA ± 1.4 pA, n= 8 cells from 4 mice; non-PV-IN MECS sEPSC amplitude, 16.4 pA ± 1.1 pA, n = 8 cells from 4 mice). Note the scaling factors (X10 or /10) for several parameters to fit on the same Y axis. Statistical analysis was carried out using a Student's t-test. * $P < 0.05$. Error bars represent s.e.m. (**f**) Cumulative probability plot for the amplitudes of all sEPSC events from all recordings obtained in PV-INs and non-PV-INs for control and MECS conditions as indicated (for PV-INs $P < 0.01$ for control vs MECs, K-S test).



Supplementary figure 9. MECS increases GluR4 levels *in vivo* (**a**) Representative fluorescence immunohistochemical staining of control (left) and MECS stimulated (right) sections in the dentate gyrus region of the hippocampus. Sections were labeled using antibodies against GluR4 (green) and Parvalbumin (red). Scale bars represent 50 µm. (**b**) Summary of the data shown in **a**. All values are presented as a mean GluR4 intensity and were normalized to WT Control samples (WT Control, 100% ± 6.07%, n = 24 cells; WT MECS, 156.46% ± 8.65%, n = 23 cells; *Narp*$^{-/-}$ Control, 123.72% ± 4.89%, n = 21 cells; *Narp*$^{-/-}$ MECS, 91.99% ± 6.69%, n = 21 cells). Statistical analysis was performed using a nonparametric one-way ANOVA test. *** $P < 0.001$ vs WT Control. Error bars represent s.e.m.



Supplementary figure 10. MECS induces a negative correlation of sEPSC amplitude and decay time constant in WT but not *Narp*$^{-/-}$ mice. (**a**) plot of sEPSC amplitude vs. decay time constant for control and MECS treated WT mice. Each point represents a single cell recording and the corresponding line is a linear regression fit of the data (r= 0.2 for control and –0.47 for MECS). (**b**) same as in **a** but for *Narp*$^{-/-}$ mice (r = –0.2 for control and 0.65 for MECS).



Supplementary figure 11. Kindling induces Narp expression (**a**) Representative fluorescence immunohistochemical staining of unkindled (left) and kindled (right) sections in the CA3 region of the hippocampus. Sections were labeled using antibodies against Narp (green) and Parvalbumin (red). Scale bars represent 100 µm. (**b**) Representative western blot showing levels of Narp and Actin in unkindled (left) and kindled (right) WT mice forebrains. (**c**) Summary of the data shown in **b**. All values are presented as a ratio of total Narp intensity/total Actin intensity and were normalized to unkindled samples (Unkindled, 100% ± 39.10%, n = 3 mice; Kindled, 238.50% ± 47.68%, n = 3 mice). Statistical analysis was performed using an unpaired T-test. * $P < 0.05$. Error bars represent s.e.m.